

## **SUPPLEMENTARY INFORMATION**

### **New insights into the ORF2 capsid protein, a key player of the hepatitis E virus lifecycle**

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**Supplementary Figure 1: Identification of the N-terminus of the ORF2c protein.** ORF2c proteins were immunoprecipitated with an anti-ORF2 antibody (4B2), denatured and incubated or not with N-succinimidylloxycarbonylmethyl tris (2,4,6-trimethoxyphenyl) phosphonium bromide (TMPP), which binds specifically to the N-terminus of intact proteins. Proteins were resolved by SDS-PAGE, digested in-gel with trypsin or AspN and analyzed by nanoLC-MS/MS. (A) Peptide covering is highlighted in grey on the sequence. Ser<sup>102</sup> in bold corresponds to the first aa of ORF2c that was identified by TMPP labeling. (B) and (C) MS/MS spectrum of N-terminal peptides of the ORF2c protein. (B) Tryptic peptide obtained from TMPP-labeled ORF2c protein. (C) AspN peptide obtained from TMPP-labeled ORF2c protein. +572 corresponds to the TMPP mass increment following TMPP labeling.

**Supplementary Figure 2: Specificity of the anti-ORF2 1E6 antibody.** (a) Detection of ORF2 protein by WB using the 1E6 antibody on supernatants and cell extracts of PLC3/HEV cells and non-transfected PLC3 cells. (b) Uncut gels are displayed.

**Supplementary Figure 3: Characterization of wt and mutant ORF2 proteins by western blot (WB).** For each panel showing western-blotting results, uncut gels are displayed.

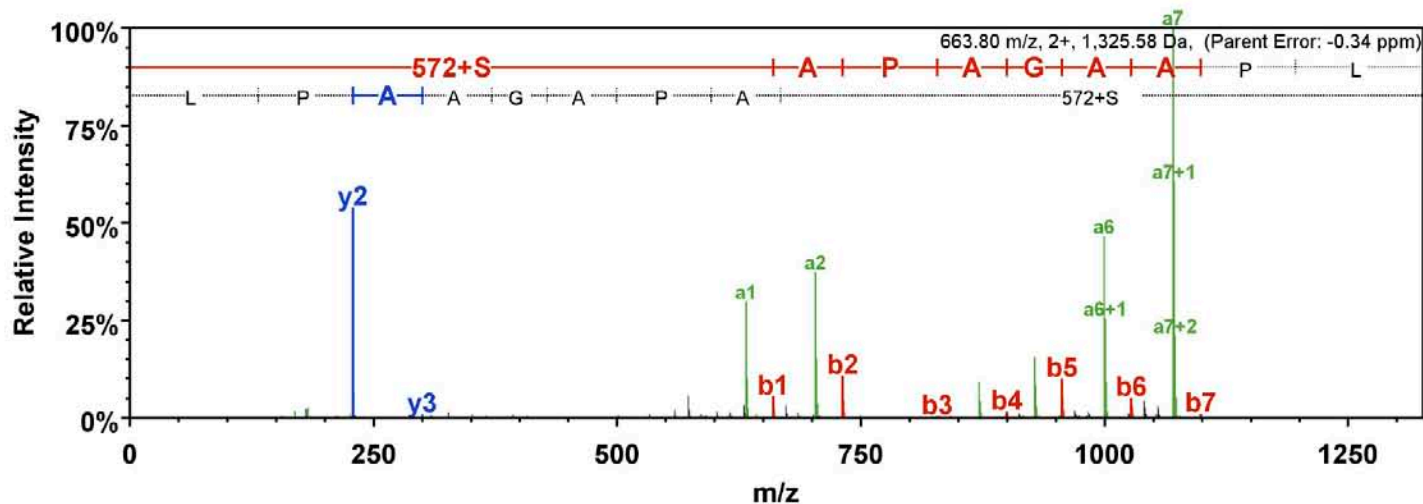
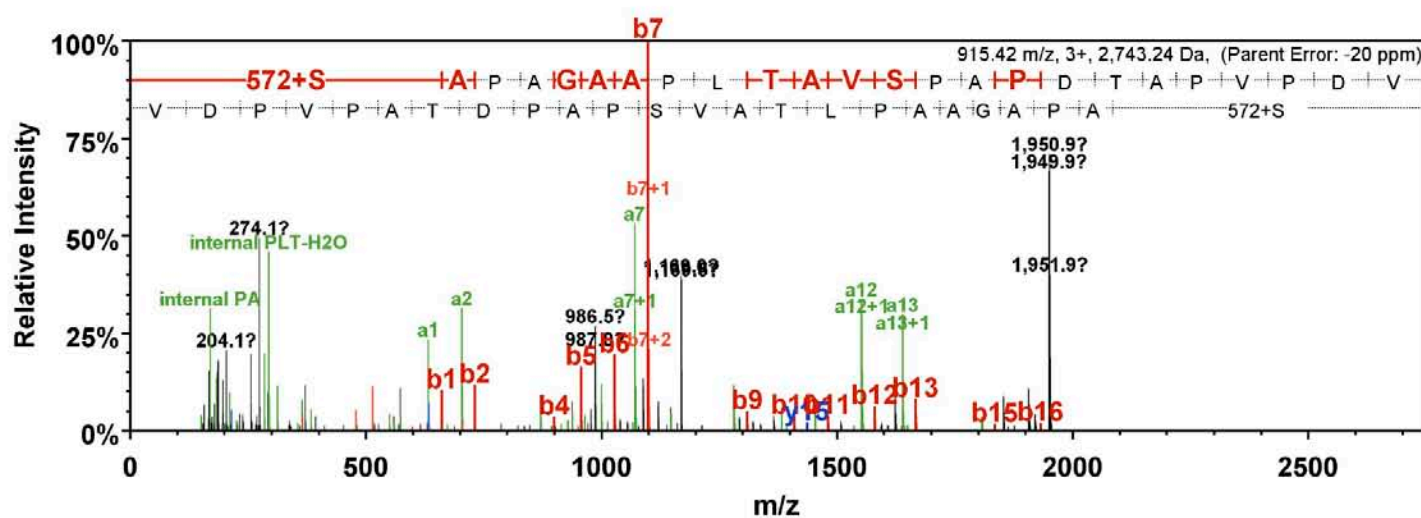
**Supplementary Figure 4: Impact of mutations of ORF2 protein N-glycosylation sites on antibody recognition.** For each panel showing western-blotting results, uncut gels are displayed.

**Supplementary Figure 5: Impact of mutations of N-glycosylation sites on ORF2 protein nuclear localization.** For each panel showing western-blotting results, uncut gels are displayed.

**Supplementary Figure 6: Impact of mutations of ORF2 protein N-glycosylation sites on particle density.** For each panel showing western-blotting results, uncut gels are displayed.

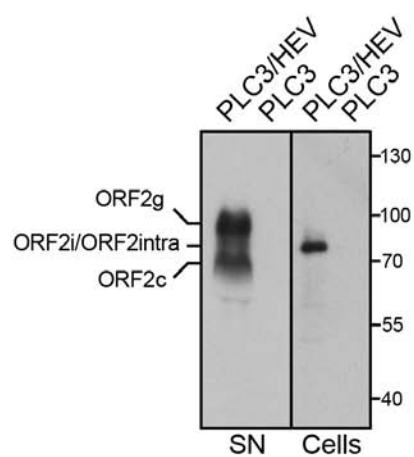
**a****ORF2c**

		Signal peptide							
1	MCPRVLLLF	FVFLPMLPAP	PAGQPSGRRR	GRR	GGAGGG	FWGDRVDSQP		50	
51	FALPYIHPTN	PFAADIVSQS	GAGTRPRQPP	RPLGSAWRDQ	SQRPSAAPRR			100	
101	R <sup>ORF2c</sup> SAPAGAAPL	TAVSPAPDTA	PVPDVDSRGA	ILRRQYNLST	SPLTSSVASG			150	
151	TNLVLYAAPL	NPLLPLQDGT	NTHIMATEAS	NYAQYRVVRA	TIRYRPLVPN			200	
201	AVGGYAISIS	FWPQTTTTPT	SVDMNSITST	DVRILVQPGI	ASELVIPSER			250	
251	LHYRNQGWRS	VETTGVAEEE	ATSGLVMLCI	HGSPVNSYTN	TPYT <sup>ORF2c</sup> GALGLL			300	
301	DFALELEFRN	LTPGNTNTRV	SRYTSTARHR	LR <sup>ORF2c</sup> RGADGTAE	LTTTAATRFM			350	
351	KDLHFTGTNG	VGEVGRGIAL	TLFNLADTLL	GGLPTELISS	AGGQLFYSRP			400	
401	VVSANGEPTV	KLYTSVENAQ	QDKGITIPHD	IDLGDSRVVI	QDYDNQHEQD			450	
451	RPTPSPAPSR	PFSVLRANDV	LWLSLTAAEY	DQATYGSSTN	PMYVSDTVTF			500	
501	VNVATGAQAV	ARSLDWSKVT	LDGRPLTTIQ	QYSKTFYVLP	LRGKLSFWEA			550	
551	GTTRAGYPYN	YNTTASDQIL	IENAAGHRVA	ISTYTTSLGA	GPASISAVGV			600	
601	LAPHSALAVL	EDTVDYPARA	HTFDDFCPEC	RTLGLQGCAF	QSTIAELQRL			650	
651	KTEVGKTRES							660	

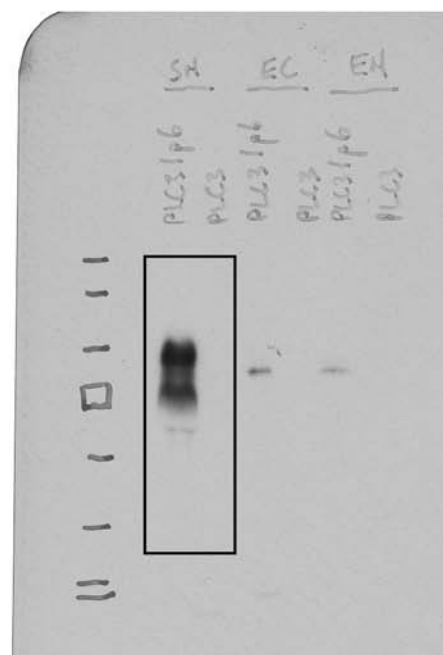
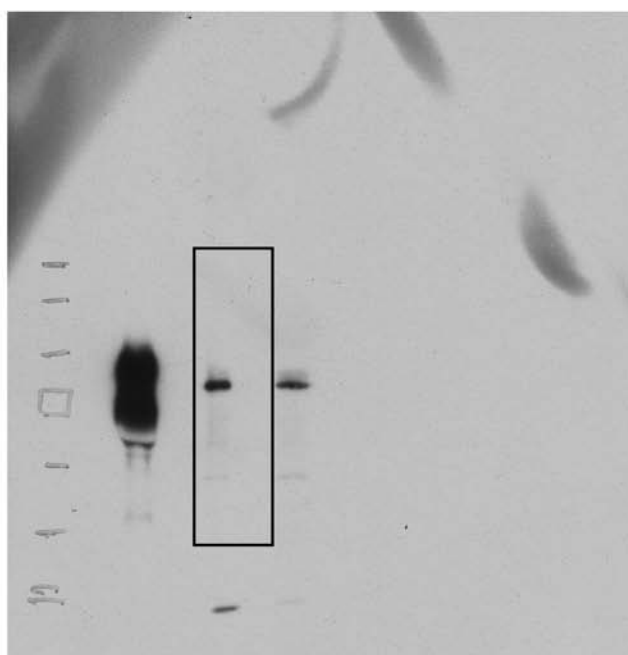
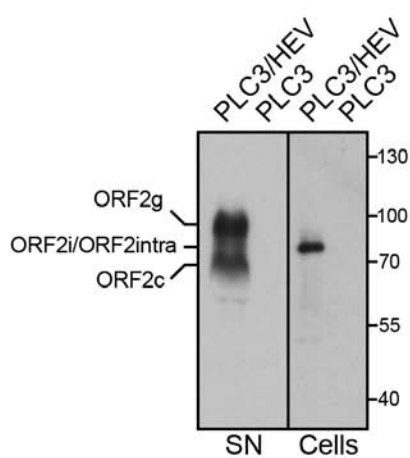
**b****c**

Supplementary Figure 1

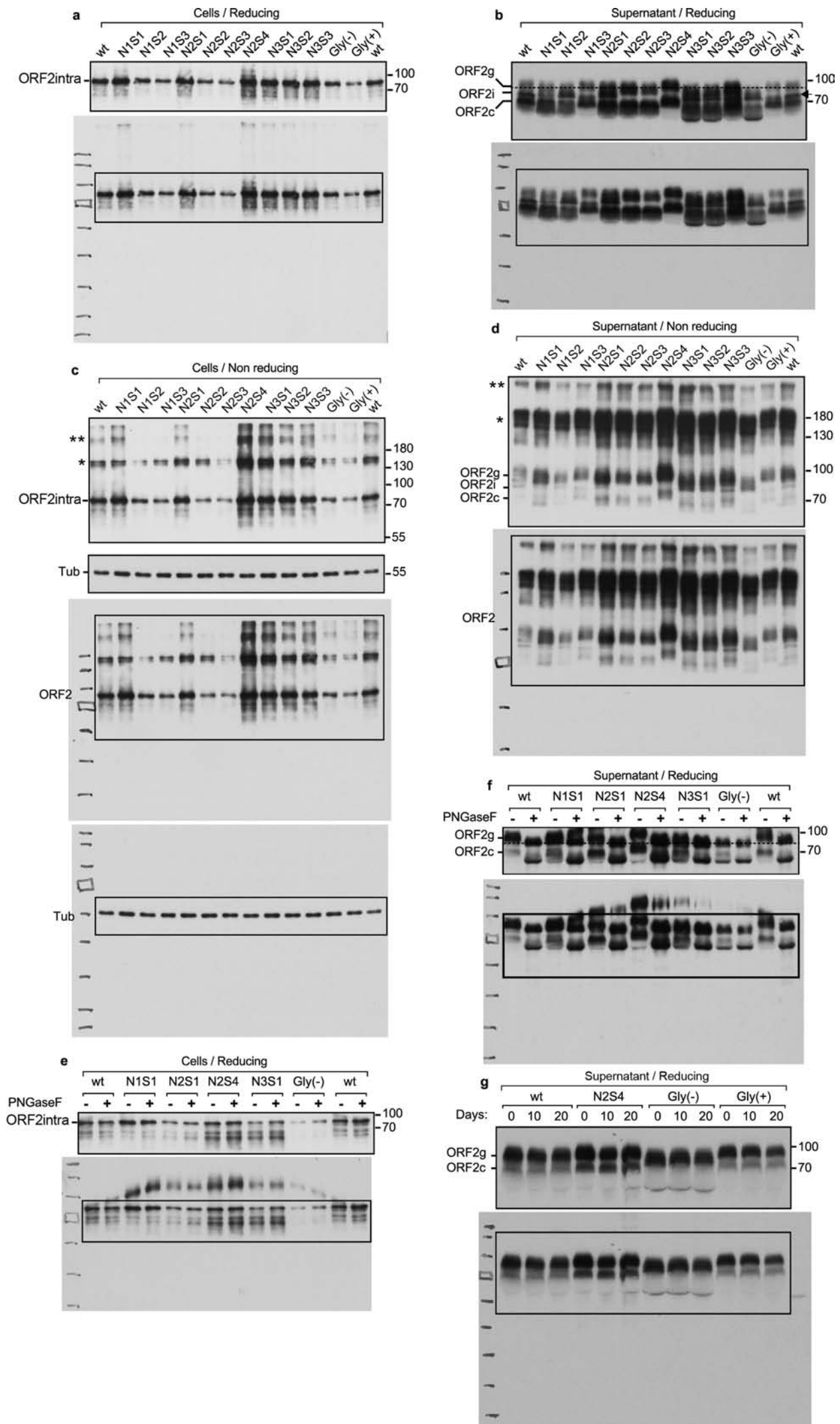
**a**



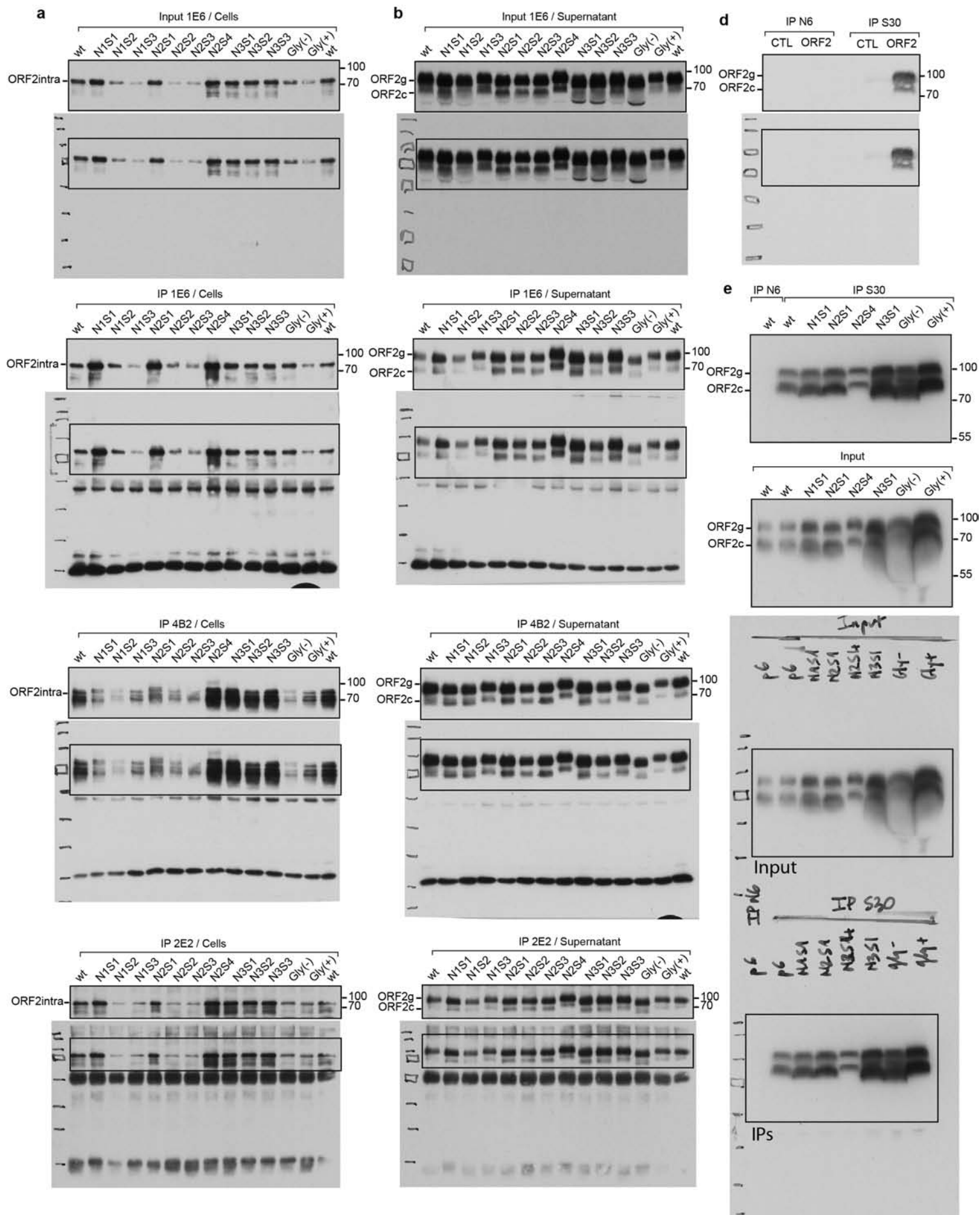
**b**



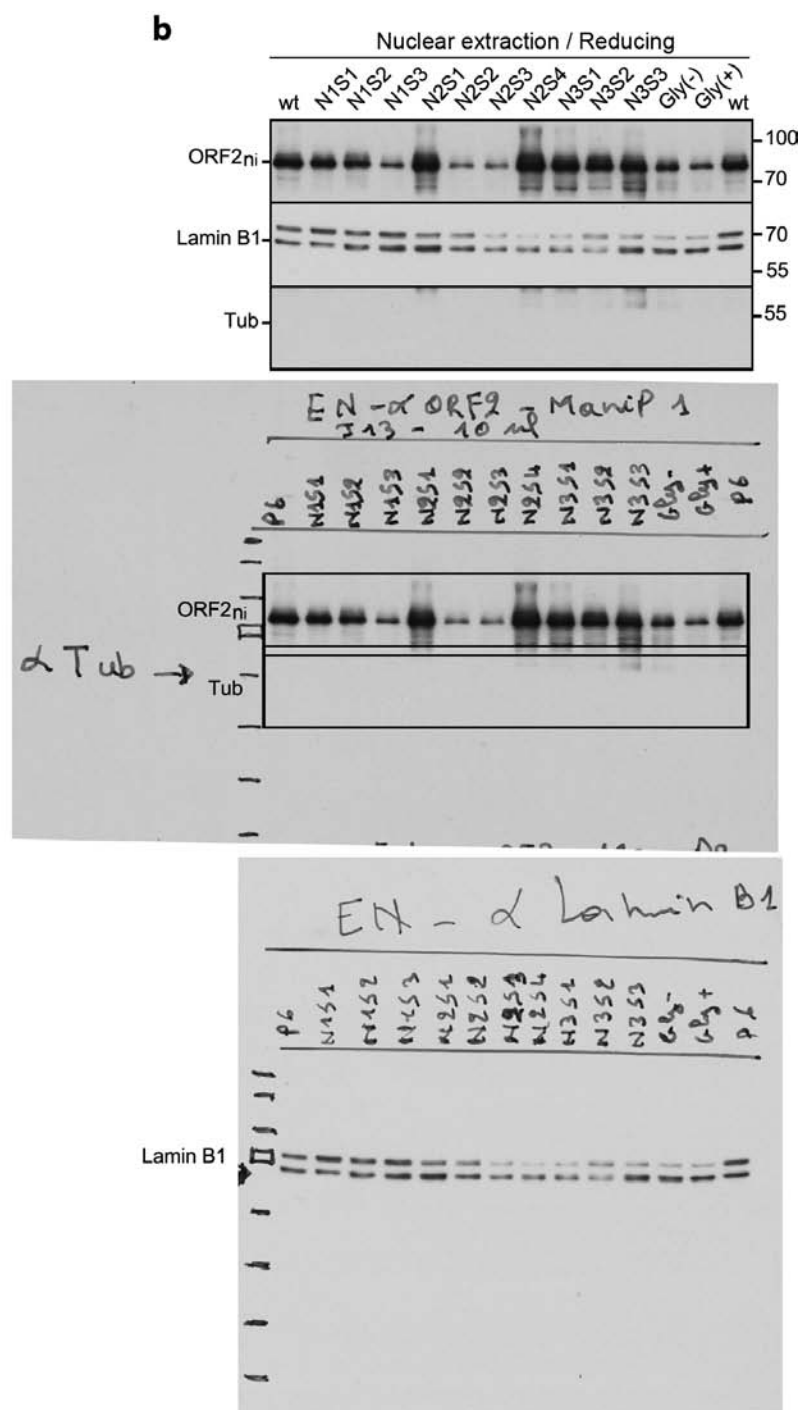
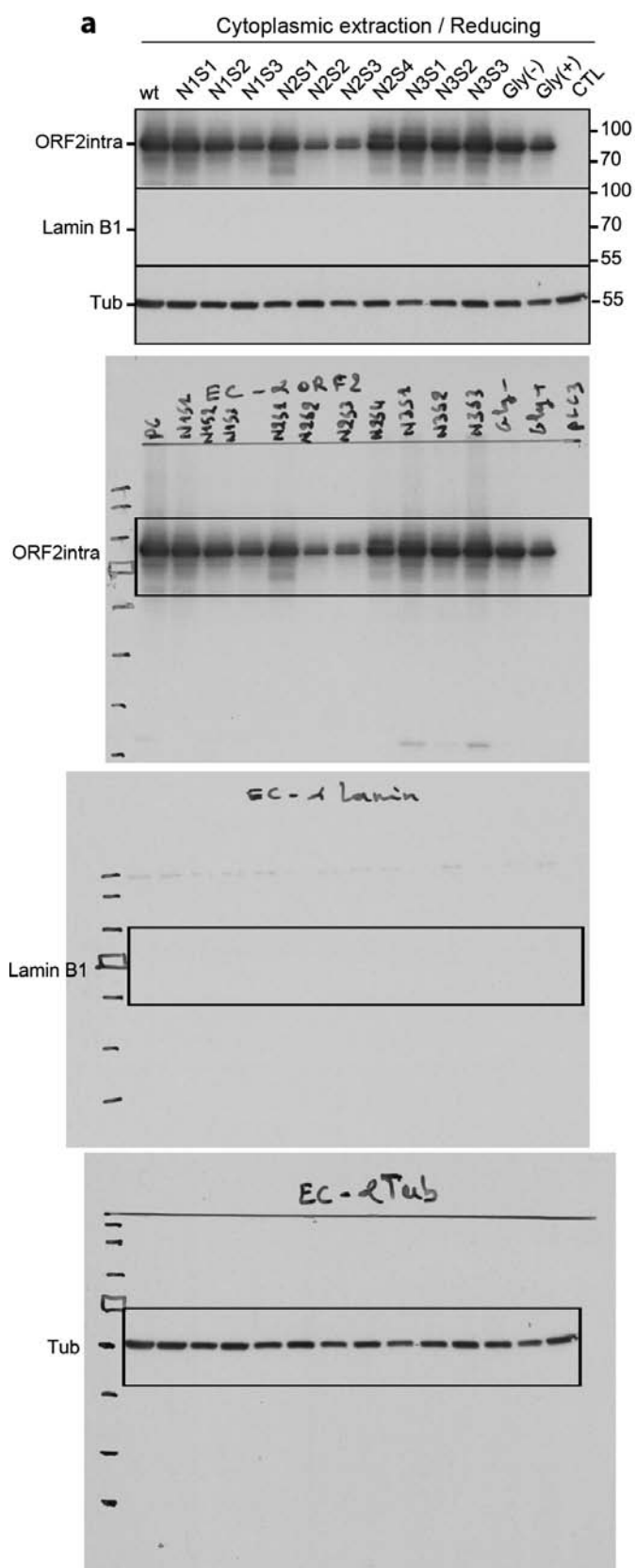
Supplementary Figure 2



Supplementary Figure 3

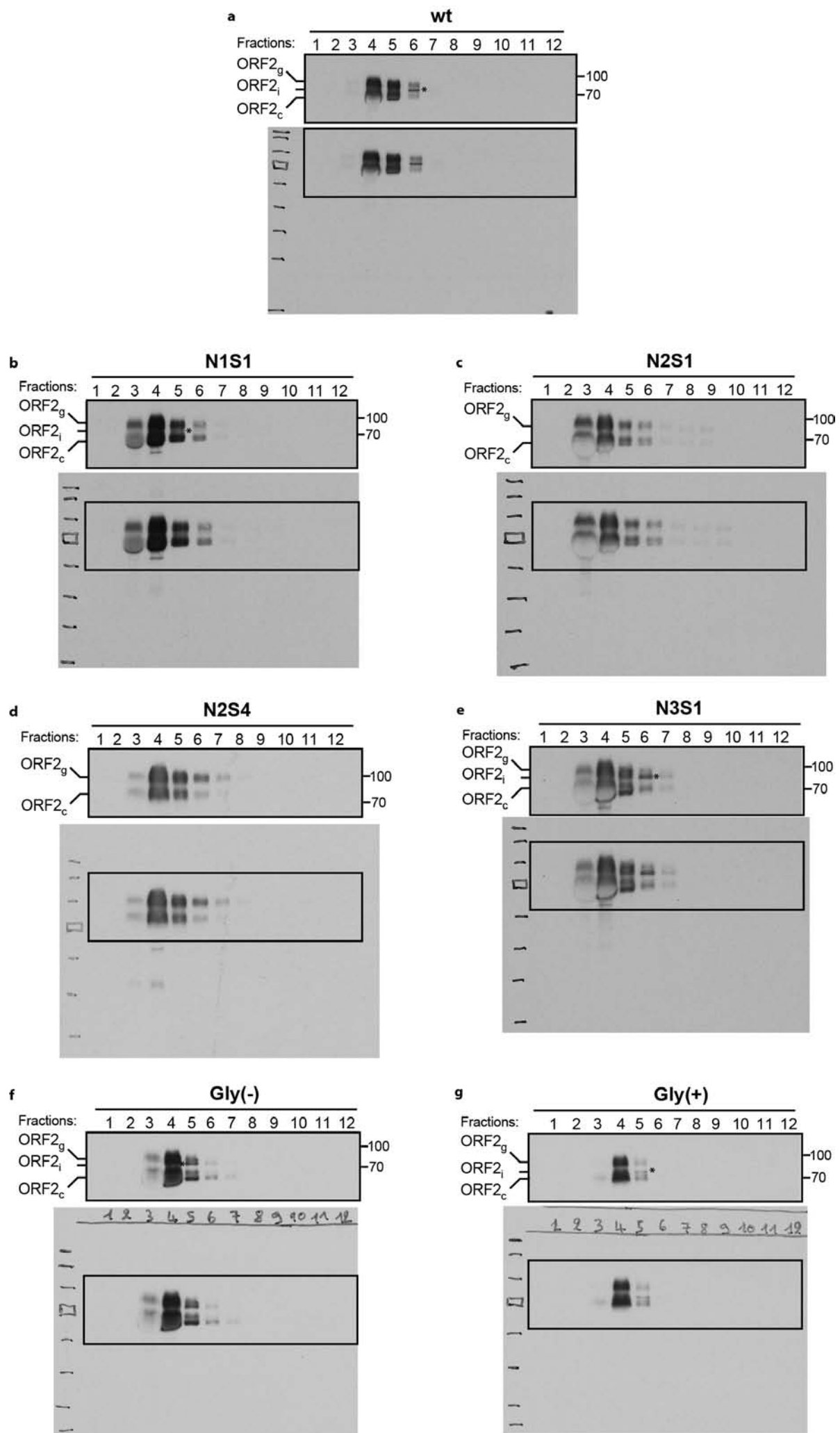


Supplementary Figure 4



Supplementary Figure 5





Supplementary Figure 6